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Role of Organic Matter in Microbial Transport during Irrigation with Sewage Effluent

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ABSTRACT

Reduction of migration of fecal coliforms (FC) and streptococci (FS) by limiting the leaching in effluent-irrigated soil was tested in lysimeters packed with quartz sand without or with added biosolids compost or with one of two clayey soils. The 200-L, 70-cm-deep lysimeters were either planted with a *Eucalyptus camaldulensis* or an Oroblanco citrus tree (in the sand only), or not planted. The *Eucalyptus* was irrigated with oxidation pond effluent (OPE) and the Oroblanco with mechanical-biological treatment plant effluent (MBTPE). The leaching fraction (LF) ranged from 0.2 to about 1.0, and the residence time (RT) from under 1 to 40 d. The *Eucalyptus* was also tested under intermittent leaching (RT 11–20 d) and deficit irrigation (without leaching for about 6 mo) regimes. Under MBTPE irrigation there was little or no leaching of FC and FS. Under OPE irrigation at LF 1 without a *Eucalyptus* there was little or no bacterial leaching at irrigation rates below 40 L d^{-1} per lysimeter (RT $\geq$ 0.8 d). Bacterial counts in the leachate were substantial in the presence of a *Eucalyptus* tree under LF 0.2 and intermittent leaching regimes, and when sand-packed unplanted lysimeters received OPE effluent at $>$45 L d^{-1}. Bacterial recovery peaked at LF 0.2, at up to 45% of the input level. At LF 1 (RT 0.6–2.8 d) and with intermittent leaching the recoveries were minute. Bacterial counts in the washout from the deficit-irrigated lysimeters were typical of nonpolluted soils. The bacterial concentration and recovery patterns in the leachate mostly matched the organic carbon (OC) load in the irrigation water, and its concentration and bioavailablity in the leachate. We related the leaching patterns of the fecal bacteria to their relative reproduction and die-off rates, and to the dependence of their regrowth on available carbon sources.

Land application of sewage effluent is increasing in many parts of the world, for agricultural use as well as disposal purposes (Feigin et al., 1991; Oron et al., 2001; Kamizoulis et al., 2003). One of the most problematic aspects of irrigation with secondary effluent is the fate of pathogenic microorganisms in the soil–plant system (Shuval, 1991; Westcot, 1997). The survival and transport of effluent-borne bacteria in soils are determined by the physical, chemical, and biological properties of both the soil and the bacteria (Gerba et al., 1975; Frankenberger, 1985; Pescod, 1992; Oron et al., 2001). Most published reports on effluent irrigation indicated that pathogenic and indicator fecal bacteria were eliminated from soils or from stream water within days or weeks after application. Survival periods were inversely related to ambient temperatures and solar irradiation, and directly related to soil moisture content, pH, and clay and organic matter contents (van Donsel et al., 1967; Gerba et al., 1975; Howell et al., 1996; Oron et al., 2001). These attenuation processes provide the basis of the World Health Organization guidelines (WHO, 2006) for safe reuse of wastewater for unrestricted irrigation of freshly edible crops. In light of the requirement for pathogen reduction of 6 to 7 orders of magnitude, the upper limit for fecal coliforms in the supply water is 10^3 per 100 mL (or 10^4 per 100 mL in the case of drip irrigation). The Israeli legislation is based on similar reasoning, and mandates the use of barriers to pathogen transfer to compensate for the permissible lesser pathogen removal at the wastewater treatment stage (Fine et al., 2006).

Transport of bacteria in the soil profile largely depends on the mutually opposing processes of convective transport and attachment of bacteria to the solid phase. Capture and adhesion of bacteria to soils depends on bacterial properties, e.g., size and cell wall properties; soil structure, porosity and mineralogy; soil solution composition and ionic strength; and the rate of soil leaching (Cushman, 2000; Maier et al., 2000). Mubiru et al. (2000) showed that the decay of two *Escherichia coli* strains in two loamy soils was faster in the more clayey one, probably because of differences in the soil water contents, but Stoddard et al. (1998) found that well structured field soil allowed rapid transport of fecal bacteria into lysimeters 60 cm below the soil surface. Smith et al. (1985) used soil columns to show that bacterial transport was better in a well structured soil than in a disturbed one, and Cushman (2000) concluded that simple straining and sedimentation of fecal bacteria in irrigated field soils were of minor importance in removal of bacteria from irrigation water, because the size of most effluent-borne bacteria is less than 5% of that of the soil particles (sand grains or soil aggregates), and the neutral buoyancy of bacteria prevents sedimentation. However, soil tillage was able to eliminate leaching of indicator bacteria from applied manure, probably because of the shearing of macropores and the disruption of the continuity of soil porosity in the plowed layer (Dean and Foran, 1992).

Yee et al. (2000) showed that the adsorption of *Bacillus subtilis* onto the surfaces of corundum and quartz was through hydrophobic and electrostatic interactions between the bacteria and the mineral surfaces. The adsorption was completely reversible and it was governed by the pH and the ionic strength of the soil solution, and the bacteria to mineral ratio; these parameters controlled the chemical speciation of the bacterial and mineral surfaces. Huysman and Verstraete (1993) reported

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Abbreviations: BC, biosolids compost; CBOD, carbonaceous biochemical oxygen demand; DOC, dissolved organic carbon; ET, evapotranspiration; FC, fecal coliforms; FS, fecal streptococci; LF, leaching fraction; MBTPE, mechanical-biological treatment plant effluent; OC, organic carbon; OPE, oxidation ponds effluent; RT, residence time.

the almost immediate removal of bacteria (*S. fecalis* and *E. coli*) from 150 m*M* NaCl suspensions that were applied to the top of sand and clay loam soil columns; the extent of bacterial removal was directly related to the hydrophobicity of the cell walls of the various strains studied. McCaulou et al. (1994) suggested that although hydrophilic bacteria attached to a matrix of a porous medium, i.e., quartz or quartz coated with organic matter, more slowly than did hydrophobic ones (both types being gram negative) once binding had occurred, the former were detached more slowly than the latter. Even small amounts of organic matter could strongly enhance the binding of microorganisms to solid surfaces (Ryan and Gschwend, 1990).

Binding and transport of actively growing microorganisms in soils was found also to depend on their metabolic activity (Cushman, 2000; Maier et al., 2000), and this was true for both motile and non-motile bacteria. In fact, the motility of *Pseudomonas florescens* enabled the cells to avoid sticking to sand grains in columns that were perfused at low fluid velocities (Camesano and Logan, 1998). Adhering bacteria could be detached from soil surfaces because of enzymatic degradation of adhesive polymers or because of chemical alterations to the bacterial surface properties caused by a changing nutritional environment. The ability of bacteria to transfer between aqueous and solid phases enables them to utilize nutrients in both phases (Griffith and Fletcher, 1991). However, Dawson et al. (1981) found that starvation enhanced adhesion to solid surfaces, whereas Wrangstadh et al. (1990) found that in a marine Pseudomonad it enhanced active detachment by synthesis of specific polymers.

Stotzky (1997) hypothesized that fast die-off of allochthonous bacteria, including fecal bacteria, was due to their poor adaptation to the 'hostile' soil environment. Thus, antagonistic soil biota, including protozoa, nematodes, parasitic bacteria, fungi, and phages, play an important role in the elimination of pathogenic and indicator microorganisms in the effluent itself (Westcot, 1997), in river water (Hendricks, 1971), and in the soil (Gerba et al., 1975; Recorbet et al., 1992; Bardgett and Griffiths, 1997). Habte and Alexander (1978) demonstrated the important role of reproduction in the ability of bacteria to maintain themselves in the presence of protozoa. In a lysimeter study on the migration of fecal bacteria in the field soil profile, following manure application, Stoddard et al. (1998) concluded that there was significant fecal bacteria regrowth (or reproduction), which mitigated the bacterial die-off to some extent. Tate (1978) demonstrated the important role of protozoa in the decline of *E. coli* inocula in a sandy soil and a Histosol, and concluded that regrowth of the coliforms extended their survival in the organic soil. Plant root exudates and debris are also important nutrient sources that support growth of soil microorganisms, sometimes even to the extent of selection of microbial communities through their differing responses to subtle differences in the types and amounts of compounds that are released (Hutsch et al., 2002). Hence, Harvey (2005) concluded that column- and field-scale tests might fail to determine the conditions for transport of the bacteria, because such tests often are not designed to assess the possible influence of the soil ecological environment on the fate of microorganisms, including its effect on rates of reproduction and predation, and availability of nutrients.

Effluent irrigation can change the nutritional, physicochemical, and biological properties of the soil, all of which might affect the fate of the fecal bacteria. In the present study we simulated the recycling and disposal of secondary effluent by using it to irrigate trees. We examined the effects on the extent of leaching of the fecal coliforms and streptococci under effluent irrigation of: (i) soil properties; (ii) effluent quality; (iii) the presence of a tree and the leaching regime; and (iv) application of biosolids compost.

MATERIALS AND METHODS

Lysimeter Construction

Data were collected in a lysimeter setup comprised of 48 200-L lysimeters in six treatments. The lysimeters were mounted on metal frames (3.0 × 0.6 × 0.6 m) in groups of three. Each lysimeter was lined with 0.1-mm-thick polyethylene. A 0.1-m layer of limestone pebbles at the bottom of the lysimeter, on top of the liner, was covered with a nylon net (1-mm mesh size) on top of which was a 0.7-m column of sand or soil. The surface area of the soil in the lysimeter was 0.25 m^2. A drainage device (5 cm long, 20-mm i.d.) was fitted to the bottom of the container and the liner.

Soils and Biosolids Compost

Lysimeters were packed with dune quartz sand or the A horizon of one of two clayey soils (Table 1) that were collected in the Judean Hills, Israel. The soils were a deep variant of Terra Rossa (a non-calcareous Red Mediterranean clay—a clayey, mixed, thermic, Vertic Palexeralf), and a deep variant of a Rendzina (colluvial-alluvial, calcareous dark-brown clay—a clayey, montmorillonitic, thermic, Calcic Haploxeroll).

Table 1. Properties of the soils used in the study.

Soil	Mechanical composition			CEC†	Exchangeable cations			V_θ‡	OC§	Carbonates (as $CaCO_3$)
	Sand	Silt	Clay		Ca & Mg	Na	K			
	g kg^{-1}				$cmol_c$ kg^{-1}			L $lysimeter^{-1}$	g kg^{-1}	
Dune sand	900	75	25	1.0	2.70	0.18	0.05	25.2	3.6	23
Vertic Palexeralf	275	375	350	49	44.4	0.46	1.66	88.2	13.5	traces
Calcic Haploxeroll	300	325	375	31	33.1	0.23	0.37	78.1	23.0	420

† CEC, cation exchange capacity.
‡ The volumetric moisture contents of two unplanted replicates per treatment were measured following ample wetting and 3 d of gravitational leaching. The V_θ of the biosolids compost-treated sand is not presented.
§ OC, organic carbon.

Table 2. Relevant constituents of the oxidation ponds (OPE) and mechanical-biological treatment plant (MBTPE) effluents, and of the biosolids compost.†

Component	Units	Sewage effluent OPE (1996)	Sewage effluent MBTPE (1999)‡	Biosolids compost
				mg kg^{-1}
pH		7.76	8.0	
EC_{25}	dS m^{-1}	1.96	1.57	
OC	mg L^{-1}	192	16	210 000
$CBOD_5$	mg L^{-1}	134	6	
Total bacteria	cfu mL^{-1}	$10^{6.9}$	$10^{5.6}$	
Total coliforms	cfu– 100 mL^{-1}	$10^{5.9}$	$10^{5.9}$	
Fecal coliforms	cfu 100 mL^{-1}	$10^{5.3}$	$10^{4.9}$	
Fecal *Streptococci*	cfu 100 mL^{-1}	$10^{3.8}$	$10^{4.3}$	
Ca	mg L^{-1}	92	80	90 000
Cl	mmol L^{-1}	10.4	7.2	
$N_{Kjeldhal}$	mg L^{-1}	54	12	14 900
Na	mg L^{-1}	270	176	2700
P_{total}	mg L^{-1}	17	2.7	15 400

† EC, electrical conductivity; OC, organic carbon; $CBOD_5$ carbonaceous biochemical oxygen demand.
‡ Data were retrieved from the WWTP records (Shafdan, Tel-Aviv, Israel), except for the OC, which was measured by us.

Biosolids compost (Table 2) was added to sand-packed lysimeters at 50 and 250 g kg^{-1} (equivalent to 125 and 625 Mg ha^{-1}, respectively) by mixing it into the upper 0.2-m layer of the sand.

Irrigation and Leaching

Low-quality secondary effluent (Table 2) was pumped directly from a nearby facultative oxidation pond at the Dan Region sewage treatment plant, Israel. Effluent was applied three times daily to the soil surface of each container via two 8-L regulated drippers. The volumes of irrigation and drainage water were monitored for each lysimeter separately, once in 2 d to once a week.

Irrigation water was applied with or without leaching. Leaching from unplanted lysimeters was close to 100% of the amount of irrigation water (leaching fraction [LF] 1). Planted lysimeters were tested under three leaching treatments: (i) constant leaching of approximately 20% of the amount applied (LF 0.2); (ii) intermittent leaching, with individual irrigation adjusted to compensate only for the actual evapotranspiration, with the aim of avoiding leaching; and (iii) deficit irrigation, where irrigation was with a fixed amount of water that was below the actual evapotranspiration.

The LF 1, 0.2, and intermittent treatments were tested in 1996, and the deficit irrigation treatment was performed in 1998. In January 1998, six *Eucalyptus* trees with trunk diameter of 15 to 20 cm at base were cut out of their containers. The trees were trimmed to leave a 30-cm-long trunk piece and the uppermost part of the main root system, and the resulting stumps were replanted in new sand-packed lysimeters. Each tree was irrigated with 10 L d^{-1} and by June they had grown sufficiently to stop all drainage. In October, the water supply to each tree was reduced to 5 L d^{-1}. Irrigation continued until 27 December. On 6–7 Jan. 1999 the soil solution was displaced by flushing with 50 L of tap water, i.e., about two volumes of soil water.

Table 3 summarizes the treatments according to: (i) type of soil; (ii) addition of biosolids compost; (iii) presence or absence of a *Eucalyptus camaldulensis* tree; and (iv) soil leaching regime. Also presented for each treatment are average season evapotranspiration rates, cumulative amounts of irrigation, and the residence time of the irrigation water in the 70-cm soil profile. The season irrigation rate was 10 to 20 m as calculated by dividing the cumulative amounts of irrigation water (m^3 per lysimeter; Table 3) by the soil surface area (0.25 m^2). This extreme value indicates the intensity of the leaching of the 70-cm soil profiles. Average daily evapotranspiration rates were calculated from the irrigation and leachate volumes, which were measured every other day during this period. The method of residence time (RT) calculation was explained previously (Fine et al., 2002). Briefly, it was calculated for each lysimeter by dividing the water-holding capacity (V_θ in Table 1) by the average daily leachate volume and it was done from 1 Aug. onward. Under deficit irrigation the overall period of irrigation without leaching was about 180 d, i.e., from the cessation of spontaneous leaching (in early June) until 27 December when irrigation ceased. The lysimeters were flushed 11 d afterward. Hence, the RT which was the period of time that effluent water constituents resided (if not degraded or consumed) in the soil was between 11 and about 180 d.

The unplanted treatments were all tested in duplicated lysimeters, the planted sand-packed, biosolids-amended treat-

Table 3. Treatment variables and parameters of *Eucalyptus camaldulensis* irrigation in lysimeters. Data from the second year after planting are presented. Values are means and standard errors.

Soil	Treatment variables: Biosolids compost	Designated LF†	With w/out a tree‡	Actual LF§	Season average daily ET§	Season cumulative irrigation¶	Residence time#
	Mg ha^{-1}	%		%	L tree^{-1}	m^3 lysimeter^{-1}	d
Sand	0	100	NT	97.5 ± 0.4			0.6 ± 0.1
	0	20	T	21.5 ± 3.7	25.6 ± 1.4	4.98 ± 0.39	3.4 ± 0.8
	0	Intermittent	T	10.6 ± 2.1	18.6 ± 1.4	3.74 ± 0.14	10.6 ± 2.8
	0	Deficit	T		5–10	1.52	11–180
	625	100	NT	97.5 ± 0.9			0.9 ± 0.3
	625	20	T	23.6 ± 1.1	19.6 ± 6.2	3.87 ± 1.2	5.4 ± 1.5
	625	Intermittent	T	10.3 ± 1.5	14.9 ± 2.2	2.51 ± 0.33	19.5 ± 0.4
Vertic Palexeralf	0	100	NT	97.3 ± 1.2			2.6 ± 1.2
	0	20	T	15 ± 0.5	14.5 ± 4.7	2.34 ± 0.43	40.0 ± 8.0
Calcic Haploxeroll	0	100	NT	97.2 ± 0.0			2.8 ± 0.0
	0	20	T	30 ± 0.8	18.9 ± 2.7	4.01 ± 0.43	9.0 ± 1.0

† Irrigation at LF 0.2 was conducted for 257 days, from 23 Mar. through 5 Dec. Intermittent leaching deviated from LF 0.2 on 1 Aug., and was maintained for 126 d. Deficit irrigation was conducted for approximately 6 mo, and irrigation ceased 11 d before flushing of the lysimeters.
‡ T, with a tree; NT, without a tree.
§ Actual daily leaching fraction (LF) and evapotranspiration (ET) were calculated every other day (from 1 Aug. to 5 Dec.) for each lysimeter from the volumes of irrigation and leachate.
¶ Irrigation volumes are the cumulative amounts of effluent applied to each lysimeter during the entire irrigation season (257 d).
# Residence time (RT) was calculated for each lysimeter by dividing the lysimeter water-holding capacity by the average daily leachate (from 1 Aug. onward). In the deficit irrigation treatment, RT was defined as the time elapsed between the first and last irrigation dates and the date of soil flushing.

ments were in triplicates, and the other planted sand- and soil-packed lysimeters were in six replicates.

Lysimeters with Oroblanco Citrus Trees

In March 1998, in a similar setup to the above, sand-packed lysimeters were planted with Oroblanco (a triploid pummelo [*Citrus grandis* (L.) Osbeck] × grapefruit [*Citrus paradisi* Macf.] hybrid) grafted on sour orange (*C. aurantium*), or were not planted. Data from the second year of the experiment are presented. Irrigation was with wastewater effluent from a mechanical-biological treatment plant (Table 2) at LF 0.3 to 0.4. The 1-yr-old citrus trees absorbed about 5 L tree^{-1} d^{-1}, which resulted in RT of 8.4 d. The same amount of irrigation water was applied to the unplanted lysimeters (LF 1) with RT of 3.1 d. Leachate samples from three planted and three unplanted lysimeters were taken weekly from mid June to mid July 1999 and analyzed for chemical indices and the indicator fecal bacteria.

Water Analyses

Leachate water samples for chemical analysis and microbial counts were collected in closed bottles placed in ice boxes during a 24-h period at 1- to 3-wk intervals (Fine et al., 2002). Wastewater effluent was sampled at the same intervals. The CBOD5 (carbonaceous biochemical oxygen demand) test was applied according to Method 5210-B of the American Public Health Association (APHA) (Clesceri et al., 1989). Organic C in leachate and in effluent water was analyzed with a Formacs, combustion total organic carbon (TOC) analyzer (Skalar, De Breda, the Netherlands). The sample was acidified to pH $\leq$ 3.5 and purged with N_2 gas for complete carbonate removal before the determination of organic carbon (OC).

Fecal Bacteria Assay

Fecal bacteria in the effluent and leachate samples were analyzed on the day of collection. The leachate bottles were kept in ice boxes during the collection operation. Membrane filtration was used to collect the microorganisms, according to APHA Method 9222B (Clesceri et al., 1989), and fecal coliforms (FC) and fecal streptococci (FS) were determined according to APHA methods 9222D and 9230C, respectively. The results are expressed as colony-forming units (cfu) per 1 mL or per 100 mL.

The recoveries were calculated from the counts of bacteria in the leachate and in the effluent water and on the respective volumes. Leachate was collected continually and it was assumed that the bacterial counts in successive sampling events were representative of the period between these samplings (Fine et al., 2002). Statistical analysis was done with the Sigmastat 2.03 software package (SPSS, 1997).

RESULTS

Irrigation and Residence Time of Water in the Soil Profile

The *Eucalyptus* trees grew rather well in all the lysimeters, and the amounts of water that were applied varied according to tree size, weather conditions, soil properties, and leaching treatment as described in more details by Fine et al. (2002). The mean daily evapotranspiration rate of the 2-yr-old trees, from 1 Aug. to 5 Dec. 1996, was 15 to 26 L (Table 3), and the maximum mean daily water uptake under LF 0.2 was about 45 L per tree, i.e., nearly twice the volume of water stored in the soil profile of a sand-packed lysimeter (V_0; Table 1).

The seasonal average RT of the irrigation water in the planted sand-packed lysimeters at LF 0.2 was 3.4 d, and that in the intermittent leaching treatment was 10.6 d (Table 3). The respective average (± SE) RTs in the unplanted counterparts were 0.4 ± 0.1 and 0.8 ± 0.2 d. The mean RT for all four sand-packed unplanted lysimeters was 0.6 (Table 3). As mentioned, the RT of irrigation water constituents in the soil profile of the deficit irrigation lysimeters ranged between 11 d and 6 mo.

The seasonal average RT of the irrigation water in the profiles of the lysimeters packed with clayey soils, with and without plants, were 40 and 2.6 d, respectively (Table 3), and those in the citrus planted lysimeters were 8.4 and 3.1 d, respectively, in planted and unplanted lysimeters.

Leaching of Fecal Coliforms

Average counts of culturable FC in the leachate from the sand-packed lysimeters and from those packed with biosolids-amended sand are presented in Fig. 1A. Also presented are the recoveries of bacteria from the leachate (Fig. 1B). Whereas the FC counts in the leachates from the planted lysimeters were of the same order of

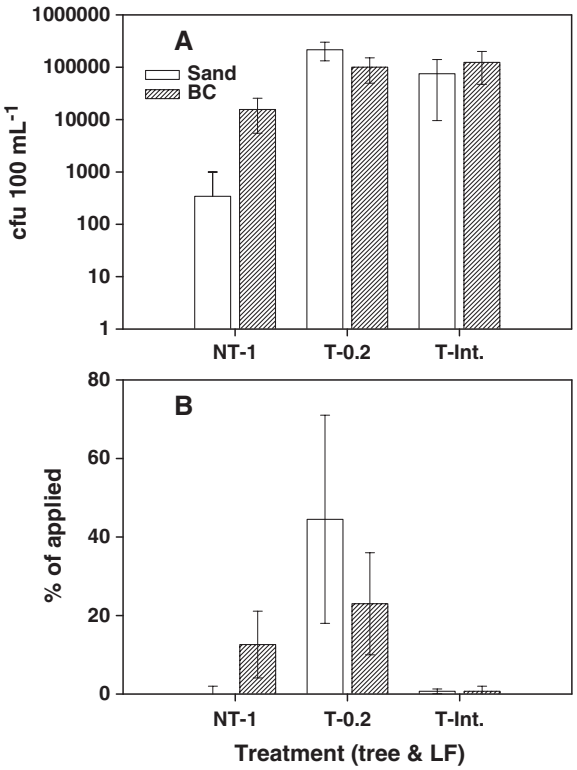

Fig. 1. Leaching of fecal coliforms (FC) from sand-packed lysimeters irrigated with oxidation pond effluent. (A) Seasonal average FC counts, and (B) recoveries are presented (vertical bars denote standard errors) in the leachate from lysimeters that were either unplanted at LF 1 (NT-1) or planted with a *Eucalyptus* tree and maintained at LF 0.2 (T-0.2) or intermittent leaching (T-Int.) regimes. The sand was either not amended or amended with biosolids compost (BC) at a rate equivalent to 625 Mg ha^{-1}.

magnitude as those in the effluent water itself (Table 2), the counts in the leachates from the lysimeters without plants were significantly lower (Fig. 1A). Moreover, the average bacterial counts in the leachates from all planted lysimeters (sand and sand-biosolids) were quite similar, whereas the counts in the leachates from unplanted lysimeters were significantly lower. The FC counts in the leachates from the unplanted lysimeters that were amended with biosolids compost were substantially higher than in those from their unamended counterparts. The counts of the fecal bacteria (FC and FS) in the washout from the deficit-irrigated lysimeters were at background levels.

The recoveries of FC in the leachates showed bell-shaped patterns when plotted against LF (Fig. 1B). At LF 1 and under intermittent leaching the recoveries were very low, at 0 to 3% of the number of bacteria applied, whereas at LF 0.2 the recoveries were higher, i.e., 20 to 45%. The FC recoveries were higher from the biosolids-amended sand under LF 1 but not under intermittent leaching (Fig. 1B).

The concentrations of FC in the leachates from the lysimeters packed with the two clayey soils were very similar to those from the sand-packed lysimeters at LF 0.2 (Fig. 2). A similar pattern was observed, with respect to the leaching regimes, in the leachates from the unplanted lysimeters, with lower concentrations of bacteria (Fig. 2A) and negligible recoveries (Fig. 2B). Substantially higher concentrations ($>10^5$ cfu per 100 mL) and recoveries (5–45% of applied bacteria) were found in the leachates from planted lysimeters that were maintained at LF 0.2. The clayey soils were not tested under intermittent leaching.

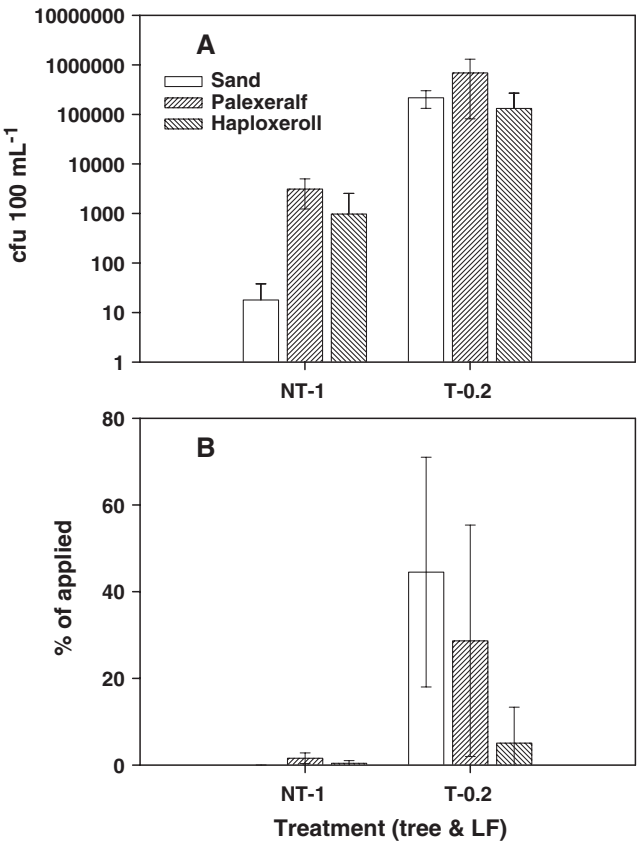

Fig. 2. Leaching of fecal coliforms (FC) from lysimeters packed with three soils and irrigated with oxidation pond effluent. (A) Seasonal average FC counts, and (B) recoveries are presented (vertical bars denote standard errors) in the leachate from lysimeters that were either unplanted at LF 1 (NT-1) or planted with a *Eucalyptus* tree and maintained at LF 0.2 (T-0.2).

Leaching of Fecal Streptococci

The pattern of leaching of culturable FS from the effluent-irrigated lysimeters followed the pattern of FC leaching very closely (Fig. 3 and 4). The bacterial counts were 1 to 2 orders of magnitude lower in the leachates from the unplanted lysimeters than in those from the planted ones. The addition of biosolids compost to the upper layer of the sand substantially increased the numbers of bacteria that leached from the unplanted lysimeters, but had no effect on those from the planted lysimeters. Also, the FS counts in the leachates from the lysimeters under the LF 0.2 and intermittent leaching regimes were very similar (Fig. 3A). The overall leaching rates of FS from the sand and from the two clayey soils were quite similar (Fig. 4A). The overall recoveries of the FS in the leachate were lower than those of the FC: up to 14% in the biosolids-amended sand treatments at LF 1, and in all sand treatments at LF 0.2 (Fig. 3B), but near zero in the intermittently leached sand treatments, both with and without biosolids, and in all the soil treatments (Fig. 4B).

Relationship between Irrigation Rate and Leaching of Fecal Coliforms

As mentioned above, the amounts of water applied to the planted lysimeters were adjusted according to the evapotranspiration rates and leaching treatments. This applied to lysimeters both with and without plants, because the latter received the same irrigation treatments as their planted counterparts. Consequently, the loads of effluent constituents, including loads of microorganisms and OC also changed over time. Figure 5A presents the leaching of FC from sand-packed, unplanted lysimeters as functions of the daily amount of irrigation water and the OC loads. It can be seen that as the daily irrigation rose above 45 L, and, in turn, the daily OC load rose above 9000 mg lysimeter^{-1}, the leaching of FC was more frequent. This daily 45-L input was equivalent to approximately twice the water-holding capacity of the lysimeter, and the daily OC load of 9000 mg was equivalent to a daily input of 3.6 mg cm^{-2} to the soil surface. It is noteworthy that even at these high water loading and soil leaching rates, some lysimeters did not discharge FC in the leachate. Unlike the unplanted lysimeters, the FC content in the leachate from planted sand-packed lysimeters showed no relationships with the daily input of irrigation water or with the OC load—the number of FC in the leachate did not change as the irrigation rate ranged from 1 to 60 L d^{-1}, i.e., as the OC input ranged from 192 up to 13000 mg lysimeter^{-1} d^{-1} (Fig. 5B).

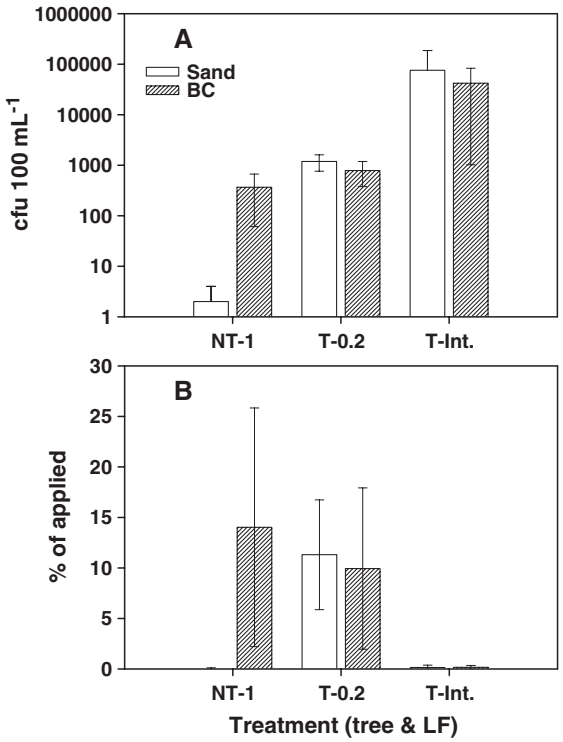

Fig. 3. Leaching of fecal streptococci (FS) from sand-packed lysimeters irrigated with oxidation pond effluent. (A) Seasonal average FS counts, and (B) recoveries are presented (vertical bars denote standard errors) in the leachate from lysimeters that were either unplanted at LF 1 (NT-1) or planted with a *Eucalyptus* tree and maintained at LF 0.2 (T-0.2) or intermittent leaching (T-Int.) regimes. The sand was either not amended or amended with biosolids compost (BC) at a rate equivalent to 625 Mg ha^{-1}.

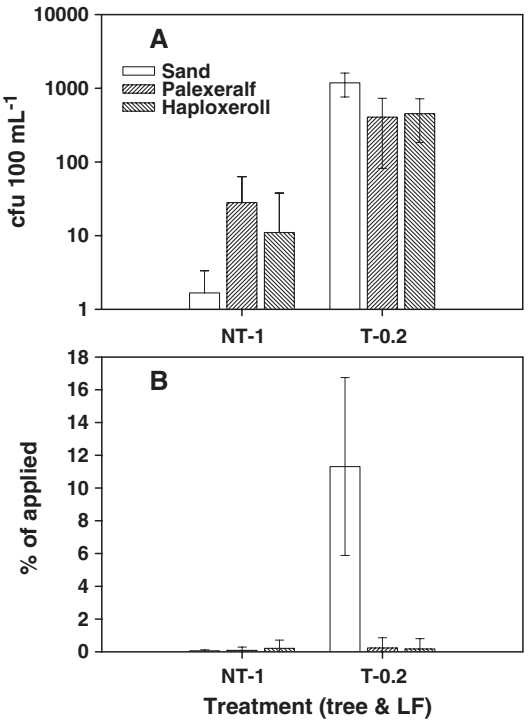

Fig. 4. Leaching of fecal streptococci (FS) from lysimeters packed with three soils and irrigated with oxidation pond effluent. (A) Seasonal average FC counts, and (B) recoveries are presented (vertical bars denote standard errors) in the leachate from lysimeters that were either unplanted at LF 1 (NT-1) or planted with a *Eucalyptus* tree and maintained at LF 0.2 (T-0.2).

Leaching of Fecal Bacteria with Respect to Leaching of Organic Carbon

The concentration of OC in the oxidation pond effluent (OPE) water was 192 mg L^{-1} (Table 2). As this water passed through the sand column within the lysimeters almost all the OC was intercepted and/or degraded, and the average OC concentration in the leachate from the lysimeters without plants was 40 mg L^{-1} (Fine et al., 2002). This is reflected in the left-hand part of Fig. 6; it is evident that little or no leaching of fecal coliforms occurred when the concentration of OC in the leachate was below about 50 mg L^{-1}.

The residual OC in the soil solution was more recalcitrant and, despite its degradation, its concentration in the leachate increased as the LF diminished. At LF 0.2 and under intermittent leaching the average OC concentrations in the leachate from the planted sand-filled lysimeters were 150 and 250 mg L^{-1}, respectively (Fine et al., 2002). In the leachate, higher counts of FC (viable cells) were associated with higher concentrations of OC (Fig. 6).

The concentration of biodegradable organic carbon in the soil solution of the effluent-irrigated soils is expected to have a direct influence on the survival of fecal bacteria capable of regrowth. A direct measure of the biodegradability of soil solution OC is the carbonaceous biochemical oxygen demand (CBOD) of the leachates. The CBOD of the OPE was 134 mg L^{-1} (Table 2). Counts of fecal bacteria in leachate samples with respect to CBOD concentrations are presented in Fig. 7. The origin position is represented by 13 measurements. It can be seen that, inasmuch as leaching of the two fecal bacteria types could occur even at zero CBOD, leaching had occurred in almost all events when the CBOD was larger than zero.

Leaching of Fecal Coliforms with Respect to Leachate Salinity

The chloride concentration in the leachate from the lysimeters—both sand- and soil-packed, and under all leaching regimes—ranged from 213 to 6840 mg L^{-1} (Fig. 8); the higher end of this range represents an approximately 20-fold increase in chloride concentration over that in the irrigation water (340 mg L^{-1}; Table 2). At chloride concentrations up to 700 mg L^{-1}, which included the unplanted lysimeters, only 12 out of 84 leachate samples had cfu mL^{-1} >1000. Thus, no bacterial leaching was clearly associated with lower soil solution salinities; it occurred mostly under more saline conditions.

Leaching of Fecal Bacteria from Mechanical-Biological Treatment Plant Effluent (MBTPE)-Irrigated Lysimeters

Leaching of the fecal bacteria was measured four times during a month starting in mid June 1999, in three

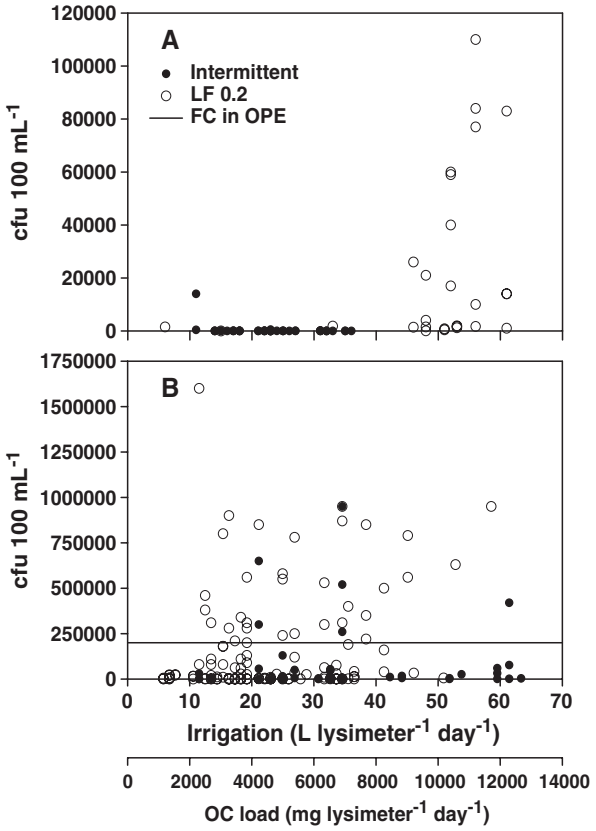

Fig. 5. Concentration of fecal coliforms in the leachate from sand-packed, oxidation pond effluent (OPE)-irrigated lysimeters in relation to the daily irrigation rate and daily OC loads. Data are presented for (A) unplanted lysimeters and (B) lysimeters planted with a *Eucalyptus* tree. The two sets of unplanted lysimeters received the same amounts of effluent as their planted counterparts (at LF 0.2 and at intermittent leaching regimes).

planted and three unplanted lysimeters. Average counts of culturable FC and FS in the leachate from the sand-packed lysimeters that were irrigated with MBTPE were usually below the detection limit (Table 4). Whereas FS

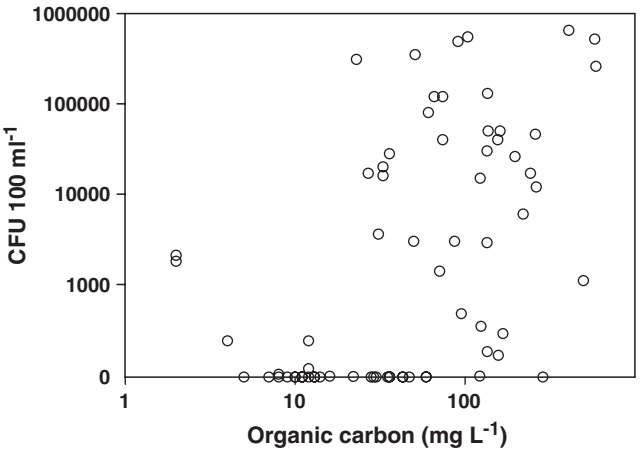

Fig. 6. Concentration of fecal coliforms (FC) in leachate from the oxidation pond effluent (OPE)-irrigated lysimeters in relation to organic carbon (OC) concentration in the leachate. Data are from all sand- and soil-packed, unplanted (at LF 1), and *Eucalyptus* planted lysimeters (at LF 0.2 and intermittent leaching), and from all the sampling events where both FC and OC data were available.

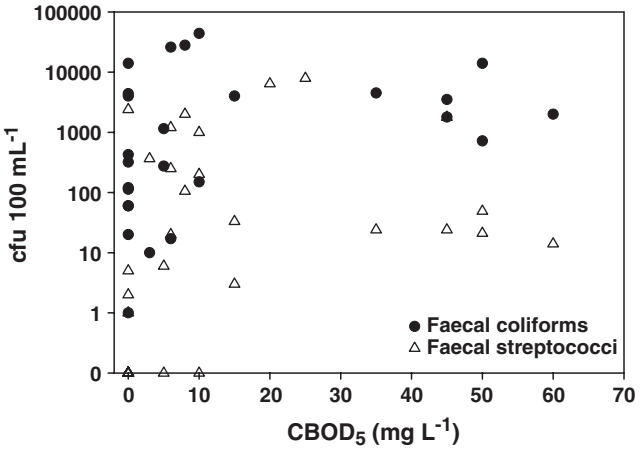

Fig. 7. Concentrations of fecal coliforms (FC) and fecal streptococci (FS) in leachate from the oxidation pond effluent (OPE)-irrigated lysimeters in relation to the carbonaceous biochemical oxygen demand (CBOD) concentration in the leachate. Data are from all sand- and soil-packed, unplanted (at LF 1), and *Eucalyptus* planted lysimeters (at LF 0.2 and intermittent leaching), and from all the sampling events where both bacteria (FC and/or FS) and CBOD data were available.

were completely intercepted in the soil, some leaching of FC did occur. Also, leaching of FC coincided with the leaching of the CBOD (Table 4). The range of CBOD values in the leachate from the effluent-irrigated lysimeters (0–11 mg L^{-1}) was similar to that of the leachate from their tap-water-irrigated counterparts (0–8 mg L^{-1}; data not shown). The average OC recoveries in the leachates from the unplanted and planted lysimeters were 74 and 91%, respectively, of the amounts in the effluent water (Table 4). The difference in OC concentration (i.e., 7 mg L^{-1}) can perhaps be attributed to exudations from

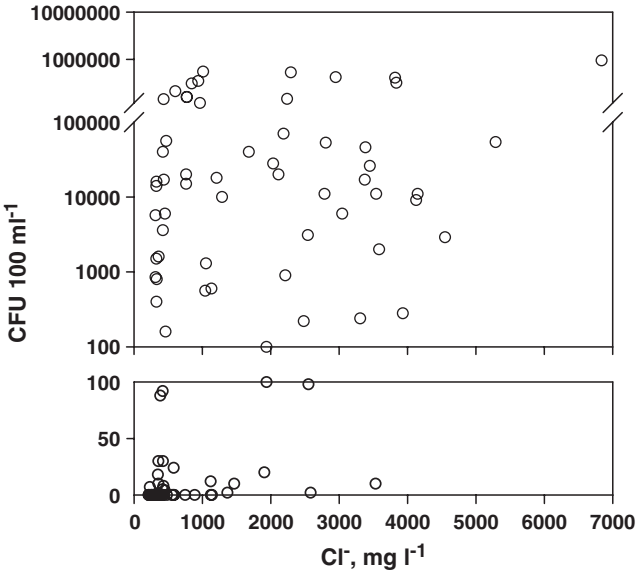

Fig. 8. Concentrations of fecal coliforms (FC) in leachate from the oxidation pond effluent (OPE)-irrigated lysimeters in relation to Cl^- concentration in the leachate. Data are from all sand- and soil-packed, unplanted (at LF 1), and *Eucalyptus* planted lysimeters (at LF 0.2 and intermittent leaching), and from all the sampling events where both FC and Cl^- data were available.

Table 4. Fecal coliforms (FC), fecal streptococci (FS), and water quality parameters of wastewater and lysimeter leachate: Oroblanco-planted and unplanted lysimeters irrigated with mechanical-biological treatment plant effluent. Leaching fraction (LF) of unplanted lysimeters is 1, and the LF of planted was 0.3 to 0.4.†

Sample	Date (1999)	EC	OC	OC Recov.	CBOD	CBOD Recov.	FC	FS
		dS m^{-1}	mg L^{-1}	%	mg L^{-1}	%	CFU 100 mL^{-1}	CFU 100 mL^{-1}
Effluent		1.51	42		16		113100	3360
No tree 1	21 June	1.95	15.9	73	10	80	129	0
	28 June	1.92	18.8	88	11	87	55	0
	5 July	2.02	14.3	64	0	0	0	0
	14 July	2.0	20.4	93	3	24	0	0
No tree 2	21 June	1.89	14.3	68	0	0	0	0
	28 June	1.92	21.7	102	3	24	245	0
	5 July	1.97	14.1	64	0	0	0	0
	14 July	1.9	17.2	81	0	0	20	0
No tree 3	21 June	1.94	10.3	48	0	0	0	0
	28 June	1.89	17.4	83	0	0	0	0
	5 July	2	11.3	51	0	0	0	0
	14 July	1.9	16.4	76	0	0	0	0
Tree 1	21 June	3.56	33.5	85	0	0	0	0
	28 June	3.53	54.8	140	0	0	38	0
	5 July	4.14	42.8	93	1	17	963	0
	14 July	3.7	49.5	119	0	0	0	0
Tree 2	21 June	9.06	25.8	26	0	0	0	0
	28 June	3.75	48.9	117	10	155	0	0
	5 July	3.89	36.6	85	3	48	0	0
	14 July	5.6	47.1	76	5	115	0	0
Tree 3	21 June	5.48	50.3	83	0	0	0	0
	28 June	6.27		86	0	0	0	0
	5 July	6.43	69.0	97	0	0	0	0
	14 July	6.1	61.0	91	0	0	0	0

† EC, electrical conductivity; OC, organic carbon; CBOD, carbonaceous biochemical oxygen demand.

the tree roots; indeed, 7 mg L^{-1} was also the difference between the average OC concentrations in the leachates from planted, fresh-water-irrigated lysimeters and from those without plants (data not shown).

DISCUSSION

We evaluated the leaching of fecal coliforms and streptococci in terms of concentrations in the leachate and cumulative amounts leached, as affected by the level of effluent treatment, the leaching intensity, the residence time of the water in the soil profile, the type of soil, the presence of a tree, and the application of biosolids compost. The data presented are from the second year of the study, after the system had probably reached a steady state with respect to the soil microflora. The main finding obtained from the irrigation with the OPE was that fecal bacteria were leached very sparsely from the unplanted lysimeters, but profusely from those that were planted with a *Eucalyptus* tree. This result seemed counter-intuitive, because the irrigation rates were the same in the two cases. It was also found that, whereas shorter residence times (<3 d) in the lysimeter without a tree and intense profile perfusion led to removal or inactivation of the fecal bacteria, when the residence in the soil profile was extended (up to 40 d in the Palexeralf) and leaching rates were slower, bacterial concentrations in the leachate were substantial—up to 45% of the amounts applied in the OPE. The recovery measurements did, however, show that although intermittent leaching did not greatly reduce the bacterial counts in the leachate, compared with leaching at LF 0.2, it markedly reduced the overall amounts of the bacteria that leached. Furthermore, under deficit irrigation the bacterial counts in the washout from the lysimeters were typical of nonpolluted soils. These reductions were consistent with more complete degradation and removal of total and readily bioavailable OC sources (Fine et al., 2002).

Physicochemical exclusion of the fecal microorganisms in soil irrigated with wastewater effluent has been widely demonstrated (Gantzer et al., 2001; Oron et al., 2001). We suggest that under the conditions of the present study, physicochemical processes played a relatively minor role in the die-off of the fecal bacteria. This was deduced from the similarity between the sand and the clayey soils in the modes and extents of microbial transport/removal, and from the bell-shaped relationship between bacteria recoveries in the leachate and the residence time. Furthermore, in the LF 1 treatments, although little chemical change occurred in the liquid phase during its passage through the soil column, the FC and FS were virtually eliminated from the leachate in these treatments. Recently, Wallach et al. (2005) reported that strong soil water repellency developed under prolonged irrigation with treated sewage effluent. This caused nonuniform distribution of soil moisture and fingered flow in the soil profile. Thus, preferential flow cannot be ruled out as a possible means for accelerated microbial transport, with or without a tree. However, leaching of the enteric bacteria under LF 1 were minimal.

Considering the possibility of channeling via decaying roots, this enhanced transport mechanism would not account for the similarity between the bacterial leaching rates of the three planted soils, nor for the significantly lower bacteria recoveries under intermittent leaching than in the LF 0.2 counterparts in sand-packed lysimeters. It should also be mentioned that under the root proliferation gradually compacted the soils in the lysimeters (which also caused the soil surface to rise).

The solution ionic strength can also be ruled out as a possible mechanism that promoted the microbial transport (Maier et al., 2000; Oron et al., 2001). Had it been a controlling factor, it would have impeded microbial transport in planted lysimeters, which had a much higher ionic strength then unplanted lysimeters. Furthermore, the reduction of the electrostatic repulsion was less relevant in the sand-packed lysimeters. Also, high ionic strength would have reduced the fecal bacterial longevity through an osmotic shock (Oron et al., 2001). Similarly, the high concentrations of organic matter in the planted lysimeters at 150 to 250 mg L^{-1} mentioned above (Fine et al., 2002) would have probably contributed to transport retardation (Ryan and Gschwend, 1990).

We attribute the leaching behavior of the tested fecal bacteria mainly to the bioavailability of OC sources in the soil profile, and to the probability that regrowth enabled the bacteria to survive predation. Irrigation with the OPE maintained a high and constant flux of OC at the soil surface, i.e., OC at 192 mg L^{-1} and CBOD at 135 mg L^{-1}. Root exudates and slough-offs in the planted lysimeters were an additional source of OC (Fine et al., 2002; Hutsch et al., 2002). We hypothesize that the relative rates of microbial die-off, on the one hand, and regrowth, on the other hand, determined whether these fecal bacteria, which are capable of regrowth (Sinton et al., 1993), would leach from the lysimeters or not. We attribute the containment of these microorganisms within the soil profile of the unplanted lysimeters to the degradation of almost all the available OC (Fine et al., 2002) and to their inability to reproduce. We suggest that the available OC was quickly degraded while passing through the soil profile, and as soon as the OC concentration became growth-limiting, the rates of die-off terminated the downward migration of the bacteria and prevented their leaching. Under irrigation with the low OC, low CBOD MBTP effluent, the fecal bacteria appeared in the leachate very infrequently and at low counts, irrespective of the presence or absence of a tree (with residence times of 8.4 and 3.1 d, respectively). This coincided with depletion of the CBOD in the leachate, and also in the soil solution, and with low OC concentrations. We suggest that with these low available OC loads in the irrigation water, the fecal bacteria were unable to reproduce sufficiently to avoid nearly complete removal.

Oron et al. (2001), who studied the survival of fecal microorganisms in a vineyard soil irrigated with low-grade OPE, found that FC survival was highest when the OC content of the soil was above 8.5 g kg^{-1}. Likewise, in the present study, the enhancement of enteric bacterial leaching by the biosolids compost in the unplanted lysimeters probably resulted from a relatively more substantial increase in the concentration of biodegradable organic matter in this treatment. In the presence of a tree, this increase probably did not enhance the reproduction of the bacteria sufficiently to enable them to leach.

Therefore, we consider that the die-off was due to predation (Habte and Alexander, 1978; Stotzky, 1997; Rønn et al., 2002), because ambient conditions probably would have favored regrowth and the ensuing leaching of bacteria. We also hypothesize that when the leaching rate was reduced with a tree present in the system, the concentrations of OC in the soil solution would have increased, which would have enhanced microbial regrowth more than it enhanced removal, because of the prolonged residence time in the soil profile.

The trees had a twofold effect on the OC in the system—they increased the concentration of the effluent-derived OC through evapotranspiration of the irrigation water, and their roots supplied additional bioavailable carbon for bacterial regrowth. The average OC concentration in the leachate from the unplanted lysimeters under OPE irrigation was 41 mg L^{-1}, i.e., about 20% of that in the irrigation water, whereas in the planted, sand-packed lysimeters (it was not measured in the clayey soil-packed ones), under the LF 0.2 and intermittent leaching regimes, it was 159 and 250 mg L^{-1}, respectively (Fine et al., 2002).

The MBTPE had a fecal bacterial count similar to that of the OPE (Table 2); however, its OC concentration was probably too low to support enough regrowth to favor net bacterial survival, and exudates from the Oroblanco roots did not reverse this trend. This further demonstrates the importance of the OC content of the effluent water in bacterial transport.

We also showed that when the overall application rate of the OPE to each lysimeter exceeded about 45 L d^{-1} (equivalent to about 240 mm d^{-1}) for a long enough period of time, fecal bacteria were leached from both the unplanted and the planted lysimeters. Evidently, at these higher input rates, the loads of fecal bacteria exceeded the physicochemical and biological filtration capacities of the soil. We attributed this to the OC-induced enhancement of replenishment through regrowth faster than removal through predation and die-off, but other mechanisms are also likely.

CONCLUSIONS

We studied the leaching of FC and FS from lysimeters with *Eucalyptus camaldulensis* planted in three soils and Oroblanco citruses planted in sand. The *Eucalyptus* trees were irrigated with OC-rich OPE and the citrus trees were irrigated with OC-depleted MBTPE. Intermittent leaching and LF ranging from 0.2 to about 1 were tested, and the residence times were from <1 to 40 d. The *Eucalyptus* was also tested under deficit irrigation without leaching for about 6 mo. The data indicated that migration of the fecal bacteria in the profiles of the three soils depended on the availability of OC. The fecal bacteria did not leach when the soil profile became depleted of OC, even under profuse irrigation and residence times shorter than 1 d. We related the dependence of the bacterial leaching behavior on the relationship between their reproduction and die-off rates, and to the role of available carbon sources (in the irrigation water and from the tree roots) in determining the bacterial reproduction rates. We showed that physicochemical parameters of the soils and the soil solutions favored the leaching of the fecal bacteria, therefore, we

assumed that their retardation was due to spontaneous die-off and predation. We further showed that under the deficit irrigation regime, the fecal bacteria counts in the soil-flush water were typical of nonpolluted soils, which indicates that disposal of low-grade secondary effluent in forest irrigation could be safe with respect to leaching of fecal bacteria.

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